

S100钙结合蛋白A4在胃癌组织中的表达及意义

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[摘要] 背景与目的: 本研究探讨S100钙结合蛋白A4(S100 calcium-binding protein A4, S100A4)在胃癌旁正常组织、慢性胃炎、肠上皮化生、腺瘤样异型增生和胃癌的组织标本中的表达及与胃癌临床病理特征及预后的关系。方法: 利用HE染色对所取胃组织标本进行病理组织学诊断; 采用免疫组织化学方法检测标本S100A4蛋白的表达; 实时定量聚合酶链反应(quantitative real-time polymerase chain reaction, qRT-PCR)法检测S100A4 mRNA表达; 蛋白[质]印迹法(Western blot)检测S100A4蛋白的表达; 采用Kaplan-Meier分析累积生存率。结果: S100A4蛋白和mRNA的表达按以下顺序逐渐增加: 胃癌旁正常组织<慢性胃炎<肠上皮化生<腺瘤样异型增生<胃癌。S100A4表达水平与肿瘤大小、浸润深度、淋巴管浸润、淋巴结转移和肿瘤TNM分期有关, 但与患者的年龄和性别无关。在较大直径、较深浸润、淋巴结转移的胃癌和肠型癌中可发现S100A4基因的过表达。采用Kaplan-Meier单因素分析显示, 弱或中度S100A4基因表达的患者与不表达的患者相比有较低的累积生存率。结论: 在胃癌旁正常组织-慢性胃炎-肠上皮化生-腺瘤样异型增生-胃癌过程中, S100A4 mRNA和蛋白表达逐渐增高; S100A4表达与胃癌肿瘤大小、侵袭深度、淋巴结转移和不良预后呈正相关; S100A4过表达参与胃癌发生和演进过程, 可以作为反映胃癌恶性程度的生物学指标。

[关键词] S100钙结合蛋白A4; 胃癌; 免疫组化; RT-PCR; 蛋白[质]迹法

DOI: 10.3969/j.issn.1007-3969.2015.06.004

中图分类号: R735.2 文献标志码: A 文章编号: 1007-3639(2015)06-0424-09

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[Abstract] **Background and purpose:** This study investigated the relationship between (S100 calcium-binding protein A4, S100A4) in chronic gastritis, intestinal metaplasia, dysplasia adenomatous, normal tissue tissue samples and expression in gastric cancer and clinical characteristics. **Methods:** HE staining of the use of gastric specimens taken for histopathological diagnosis; using immunohistochemistry to detect the expression of tissue S100A4 protein; qRT-PCR was used to detect mRNA expression of S100A4 gene; Western Blot detection of S100A4 gene encoding protein. Kaplan-Meier survival curves were used to distinguish and compare survival. **Results:** S100A4 protein and mRNA expression gradually increased in the following order: normal tissue<gastritis<intestinal metaplasia<dysplasia<carcinoma. S100A4 levels with tumor size, depth of invasion, lymphatic invasion, lymph node metastasis and lymph node metastasis (TNM) staging, but with cancer patients independent of age and gender. Intestinal type (IT) cancer showed higher than the diffuse type (DT) of cancer S100A4 expression. S100A4 gene overexpression in a larger diameter, deeper invasion, lymph node metastasis of gastric and IT cancer was found. Kaplan-Meier method using univariate analysis showed weak expression of S100A4 gene is not expressed in patients with moderate or S100A4 gene have lower survival rates compared. **Conclusion:** Upregulated S100A4 gene may play an important role in the malignant transformation of epithelial cells in the stomach, and gastric cancer growth, invasion, metastasis and prognosis were positively correlated.

基金项目: 辽宁省科技厅基金(2013225021)。

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Thus, S100A4 may be considered as a potential marker to show violations of gastric cancer. S100A4 expression in diffuse unique cancer can be used to distinguish between cancer and diffuse intestinal cancer, and can be used as a molecular characteristic distinguish two types of cancer.

[**Key words**] S100 calcium-binding protein A4; Gastric cancer; Immunohistochemistry; RT-PCR; Western blot

胃癌是我国癌症相关死亡率的第二大原因^[1], 仅次于肺癌。自从20世纪下半叶以来, 虽然胃癌的发病率及死亡率在世界范围内有下降趋势, 它仍是全球主要的健康问题^[2]。因此, 阐明胃癌的发生、发展的分子机制和可靠的生物标记物的筛选对胃癌的预防、治疗和预后评估至关重要的。

S100蛋白是钙结合蛋白, 呈酸性, 相对分子质量为 $10 \times 10^3 \sim 12 \times 10^3$, 由于其在100%饱和硫酸铵中完全溶解而命名。S100钙结合蛋白A4 (S100 calcium-binding protein A4, S100A4), 又称MTS1(转移相关基因)等, 其过表达, 已被证明可以控制细胞周期进程和调节细胞间黏附, 以及肿瘤细胞的侵袭性和转移性^[3]。S100A4基因启动子含有一个ErbB2的应答元件, 而S100A4基因的增强子和沉默子元件可被甲基化强烈影响^[4-5]。有报道称S100A4在体外结合到p53的C-末端调节结构域并抑制其磷酸化, 从而抑制了p53的靶基因的转录, 包括p21/WAF、Bax蛋白、血小板反应蛋白-1和MDM-2^[6]。S100A4还参与细胞骨架重排和细胞运动, 如F-肌动蛋白、肌球蛋白-II_A、原肌球蛋白和非肌肉肌球蛋白II的重链^[7-9]。此外, S100A4已经表明通过其对血管形成、细胞骨架完整性、基质金属蛋白酶(MMPs)的活性、肿瘤相关的转录因子和基质因子的影响, 以呈现其转移前作用^[10-11]。

本研究的目的是分析胃癌及癌前病变中S100A4基因在蛋白和mRNA水平的表达, 并进一步比较S100A4基因的表达与胃癌的临床病理特征及预后的关系。

1 材料和方法

1.1 材料

收集中国医科大学附属盛京医院2005年1

月—2015年1月胃组织标本, 内镜活检组织中收集慢性胃炎(73例), 胃黏膜肠上皮化生(86例)和腺瘤样异型增生(63例), 胃癌及癌旁正常组织标本(各348例)来源于胃手术切除组织。所有患者在手术前未接受化疗, 放疗或辅助治疗。苏木精和伊红染色(hematoxylin and eosin, HE)确认病理组织学诊断。标本的部分组织冻结在-80℃液氮中用于随后RNA和蛋白质的提取。根据国际抗癌联盟(Union Internationale Contre le Cancer, UICC)标准对胃癌标本进行TNM(tumor-node-metastasis)分期^[12], 病理组织学分型依照Lauren's分型^[13-14]的判定, 胃癌细胞淋巴管和静脉侵犯由HE染色评估, 必要时进行D2-40免疫染色和EVG染色。预后统计通过门诊复查和电话随访方式进行。

1.2 方法

1.2.1 组织芯片制备及免疫组化分析

收集2005年1月—2015年1月存档蜡块作为所需的供体蜡块, 组织标本在4%的中性甲醛溶液固定, 石蜡包埋, 切成4 μm的石蜡切片。显微镜下观察相应HE切片确定具有代表性的病变部位。选取时应避开坏死较多处。将优质石蜡和蜂蜡按100:1混合, 经多次沉淀去除杂质铸成具有较好柔韧度和硬度的蜡块。利用Tissue Arrayer对受体蜡块进行打孔(直径2 mm), 而后在选取的供体蜡块中穿取标记组织(直径2 mm), 准确放入受体蜡块的小孔中。按序依次操作, 同时作记录。将受体蜡块放入50℃温箱中烤20~40 min, 待石蜡软化, 使供体蜡块组织和受体蜡块均匀融合。石蜡切片机连续切片, 厚度4 μm, 敷贴于10%多聚赖氨酸预先处理的载玻片上, 低温保存。采用EnVision方法对胃癌及其癌前病变组织芯片进行免疫组化染色。兔抗人S100A4多克隆抗体(工作浓度1:200)购自Abcom公司。所有步骤依据产品说明进行, 并利用PBS(0.01 mol/L, pH值为7.4)替代一抗作为阴性

对照。

免疫组化染色结果判定: 免疫组化切片随机分配给两名有经验的病理学医生独立分析, 这两名病理学医生事先不了解患者的临床资料。在200的放大倍率, 来确定S100A4阳性细胞数, 根据阳性染色细胞的百分比进行半定量分级并根据评分系统分为四级: 阴性(-), 0%~5%; 弱阳性(+), 6%~25%; 阳性(++), 26%~50%和强阳性(+++)>50%。

1.2.2 蛋白 [质] 印迹法 (Western blot) 检测 S100A4 蛋白

组织蛋白提取后, 采用BCA法进行裂解液中蛋白浓度的测定, 然后进行常规的Western blot法操作。应用凝胶成像分析软件(Quantity One)进行灰度扫描, 通过目的蛋白与内参照蛋白GAPDH的积分吸光度比值表示蛋白相对表达量。

1.2.3 实时定量聚合酶链反应 (quantitative real-time polymerase chain reaction, qRT-PCR) 法检测 S100A4 mRNA

经提取总RNA后进行实时定量PCR反应, 引物序列S100A4基因正义链为5'-AGCTTCTTGGGAAAAGGAC-3', 反义链为5'-TACACATCATGGCGATGCAG-3'(引物长度均为130 bp); GAPDH基因正义链为

5'-CAATGACCCCTTCATTGACC-3', 反义链为5'-TGGAA GATGGTGATGGGATT-3'(引物长度均为135 bp)。

采用相对定量的方法, 以GAPDH基因mRNA的表达为内参基因, 依据公式 $\Delta Ct = Ct(\text{目的基因}) - Ct(\text{内参基因})$, 分别计算实验组和对照组的 ΔCt , 再依公式 $\Delta\Delta Ct = \Delta Ct(\text{实验组}) - \Delta Ct(\text{对照组})$, 计算出 $\Delta\Delta Ct$ 值, 最后计算相对表达量的差值即 $2^{-\Delta\Delta Ct}$, 其表示的是实验组目的基因的表达相对于对照组的变化倍数。

1.3 统计学处理

统计分析采用 χ^2 检验或Fisher's Exact Test比较计数资料的组间差异, Mann-Whitney Test检验比较计量资料组间差异, Kaplan-Meier生存曲线用于区分和比较生存率。Cox比例风险模型用于多因素分析。应用SPSS 10.0软件(SPSS Inc., Chicago, IL, USA)分析数据, $P < 0.05$ 为差异有统计学意义。

2 结 果

2.1 免疫组化分析S100A4的表达

S100A4蛋白的表达通过测定肿瘤细胞阳性染色的数量采用四点计分制进行评估。如图1所示, S100A4蛋白定位于胃上皮细胞, 肠上皮化生, 腺瘤样异型增生和胃癌细胞的细胞质和细

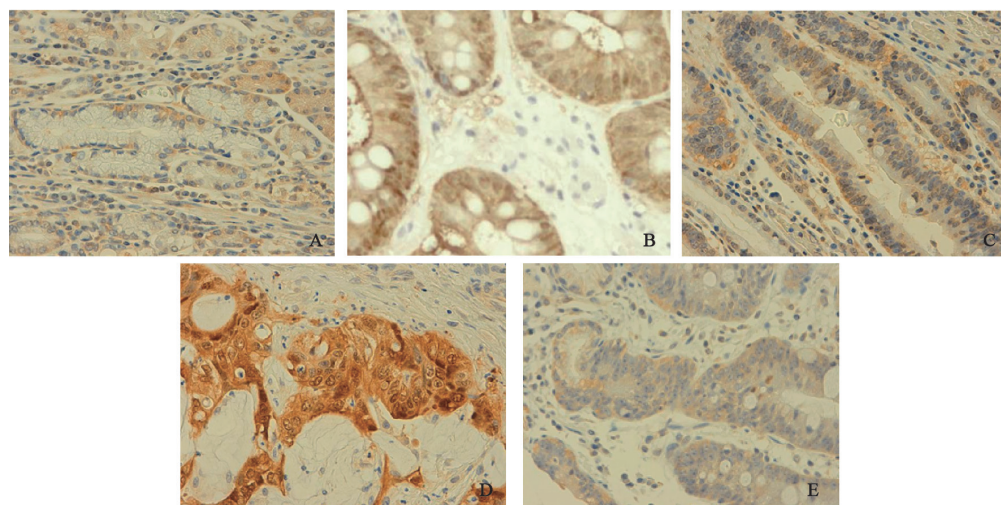


图1 胃组织中的S100A4蛋白免疫组化染色

(EnVision, $\times 100$)

Fig.1 Immunohistochemical staining of S100A4 protein showed positive expression in different gastric lesions

A: Gastritis; B: Intestinal metaplasia; C: Adenomatous dysplasia; D: Cancer tissues; E: Normal tissue.

胞核中，出现棕黄色颗粒。S100A4蛋白阳性表达率为癌旁正常组织8.3% (29/348)，慢性胃炎19.2% (14/73)，肠上皮化生23.3% (20/86)，腺瘤样异型增生34.9% (22/63)，胃癌55.2% (192/348)。基于S100A4表达的频率和密度，我们推断S100A4蛋白的表达按以下顺序逐渐增加：癌旁正常组织<慢性胃炎<肠上皮化生<腺瘤样异型增生<胃癌($P<0.001$ ，表1)。

S100A4蛋白的表达与临床病理特征之间的相关性分析，分析S100A4蛋白阳性表达与临床病理特征之间的相关性及可能影响胃癌患者预后

的因素。结果发现，S100A4基因的表达与肿瘤大小、浸润深度、淋巴管和静脉侵犯、淋巴结转移，肿瘤TNM分期($P<0.05$)呈正相关，但是患者的年龄和性别对S100A4表达无影响($P<0.05$)。S100A4基因表达在肠型(IT)胃癌高于弥漫型(DT)癌($P<0.001$ ，表2)。

2.2 qRT-PCR和Western blot分析S100A4表达与胃癌临床病理特征相关性的结果

S100A4 mRNA的表达按以下顺序上调：慢性胃炎<肠上皮化生<腺瘤样异型增生<胃癌($P<0.001$ ，图2，表3)。本研究发现在直径较大

表 1 胃组织中S100A4蛋白的表达

Tab. 1 Differential expression of S100A4 protein in gastric tissue specimens

Group	n	S100A4 expression				PR/%	P value
		-	+	++	+++		
Normal tissue	348	319	16	10	3	8.3	<0.001
Gastritis	73	59	8	4	2	19.2	
Intestinal metaplasia	86	66	13	5	2	23.3	
Adenoma	63	41	13	7	2	34.9	
Gastric carcinoma	348	156	29	62	101	55.2	

-: Negative, 0%-5%; +: Weakly positive, 6%-25%; ++: Moderately positive, 26%-50%; +++: Strongly positive, >50%; PR: Positive rate.

表 2 胃癌组织中S100A4蛋白的表达与临床病理特征的关系

Tab. 2 Association of S100A4 protein expression with the clinicopathological features of the gastric cancer patients

Clinicopathological features	n	S100A4 expression				PR/%	P value
		-	+	++	+++		
Age/year							0.277
< 55	139	67	12	23	37	51.8	
≥ 55	209	89	17	39	64	57.4	
Gender							0.183
Male	248	102	27	46	73	58.9	
Female	100	54	2	16	28	46.0	
Tumor size/cm							<0.001
≤ 4	120	84	4	11	21	30.0	
> 4	228	72	25	51	80	68.4	
Depth of invasion							<0.001
T _{is} -T ₁	33	25	2	2	4	24.2	
T ₂ -T ₄	315	131	27	60	97	58.4	
Lymphatic invasion							<0.001
-	192	116	14	20	42	39.6	
+	156	40	15	42	59	74.4	
Venous invasion							0.44
-	245	135	17	32	61	44.9	
+	103	21	12	30	40	79.6	
Lymph node metastasis							0.002
-	138	83	4	12	39	39.8	
+	210	73	25	50	62	65.2	
TNM staging							<0.001
I	6	6	0	0	0	0.0	
II	60	48	2	4	6	20.0	
III	104	50	5	15	34	51.9	
IV	178	52	22	43	61	70.8	
Lauren's classification							<0.001
Intestinal-type	203	131	7	23	42	35.5	
Diffuse-type	145	25	22	39	59	82.8	

-: Negative, 0-5%; +: Weakly positive, 6%-25%; ++: Moderately positive, 26%-50%; +++: Strongly positive, >50%; PR: Positive rate.

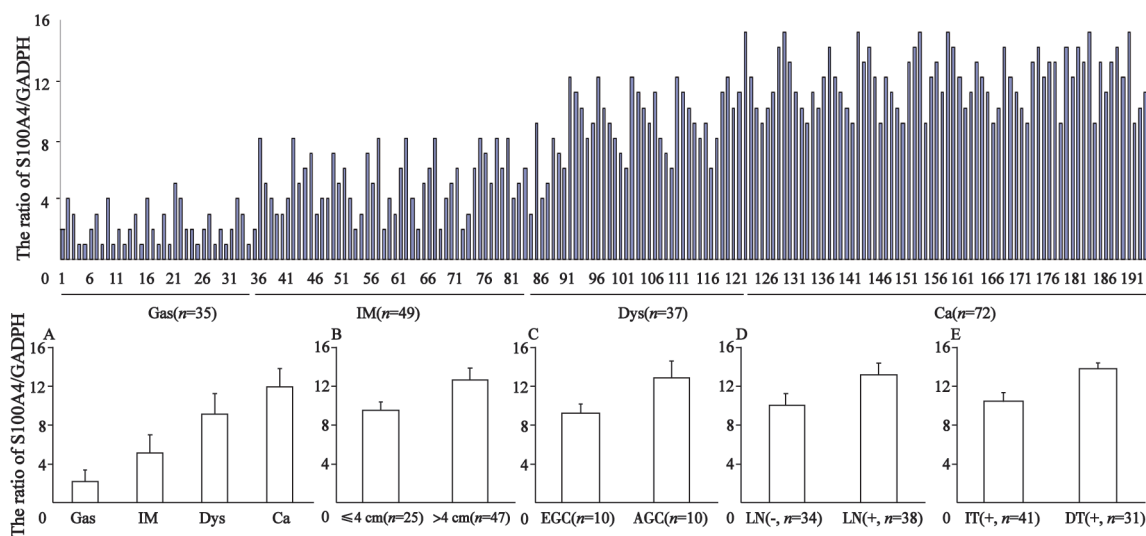


图2 S100A4 mRNA的表达水平与胃癌临床病理特征的关系

Fig. 2 Association of S100A4 mRNA expression with the clinicopathological features of the gastric cancer patients

A: S100A4 mRNA was detectable in gastritis (Gas), intestinal metaplasia (IM), dysplasia (Dys), and carcinoma (Ca) samples, as revealed by qRT-PCR analysis, and it gradually increased from Gas, IM, Dys to Ca (*: $P < 0.05$; Ca vs Gas). B: S100A4 mRNA expression was higher in gastric carcinomas that showed a larger diameter (*: $P < 0.05$; >4 cm vs ≤ 4 cm). C: Deeper invasion (*: $P < 0.05$; AGC vs EGC). D: Frequent lymph node metastasis [*: $P < 0.05$; LN(+) vs LN(-)]. E: Intestinal-type (*: $P < 0.05$; DT vs IT). Data were presented as $\bar{x} \pm s$. EGC: Early gastric cancer; AGC: Advanced gastric cancer; LN: Lymph node metastasis; IT: Intestinal-type; DT: Diffuse-type.

表3 实时RT-PCR检测胃癌组织中S100A4蛋白的表达与临床病理特征的关系

Tab. 3 Association of S100A4 protein expression with the clinicopathological features of the gastric cancer patients by RT-PCR

Clinicopathological features	n	S100A4 expression	P value
Age/year			0.258
< 55	139	0.780 4±0.105 2	
≥ 55	209	0.810 5±0.943 8	
Gender			0.196
Male	248	0.815 4±0.078 2	
Female	100	0.783 4±0.094 3	
Tumor size/cm			<0.001
≤ 4	120	0.764 3±0.095 4	
> 4	228	0.869 3±0.102 3	
Depth of invasion			<0.001
T _{is} -T ₁	33	0.650 8±0.090 6	
T ₂ -T ₄	315	0.783 9±0.082 2	
Lymphatic invasion			<0.001
-	192	0.823 9±0.081 9	
+	156	0.859 2±0.101 5	
Venous invasion			0.56
-	245	0.715 7±0.106 9	
+	103	0.823 0±0.125 3	
Lymph node metastasis			0.003
-	138	0.793 4±0.048 3	
+	210	0.940 5±0.034 5	
TNM staging			<0.001
I	6	0.634 8±0.143 6	
II	60	0.734 9±0.074 2	
III	104	0.803 9±0.082 9	
IV	178	0.856 2±0.101 9	
Lauren's classification			<0.001
Intestinal-type	203	0.647 3±0.079 2	
Diffuse-type	145	0.8345 4±0.094 9	

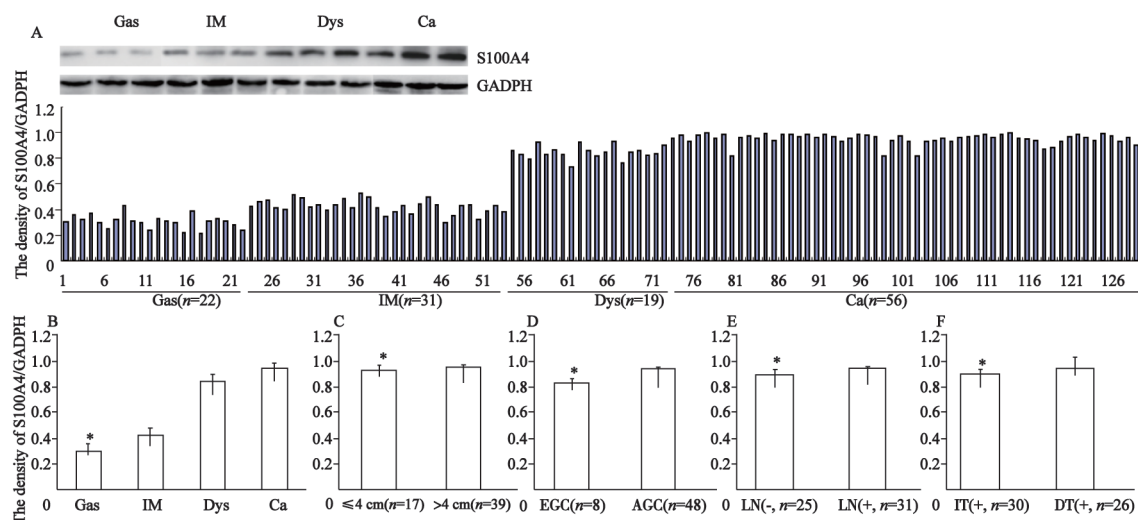


图3 S100A4蛋白的表达水平与胃癌临床病理特征的关系

Fig. 3 S100A4 protein expression level during gastric carcinogenesis and its correlation with clinicopathological features of carcinoma.

A: Using GADPH (37×103) as an internal control. B: Densitometric analysis demonstrated S100A4 expression in gastritis (Gas), intestinal metaplasia (IM), dysplasia (Dys), and carcinoma (Ca) specimens, and the S100A4 protein expression level gradually increased from Gas, IM, Dys to Ca (*: $P < 0.05$, Ca vs Gas). C: S100A4 protein expression was higher in gastric carcinomas that showed a larger diameter (*: $P < 0.05$, $> 4\text{ cm}$ vs $\leq 4\text{ cm}$). D: Deeper invasion (*: $P < 0.05$, AGC vs EGC); E: Frequent lymph node metastasis *: $P < 0.05$, LN(+) vs \leq LN(-). F: Intestinal-type (*: $P < 0.05$, DT vs IT). Data were presented as $\bar{x} \pm s$. EGC: Early gastric cancer; AGC: Advanced gastric cancer; LN: Lymph node metastasis; IT: Intestinal-type; DT: Diffuse-type.

表4 Western blot检测胃癌组织中S100A4蛋白的表达与临床病理特征的关系

Tab. 4 Association of S100A4 protein expression with the clinicopathological features of the gastric cancer patients by Western blot

Clinicopathological features	n	S100A4 expression	P value
Age/year			0.189
< 55	139	1.785 9±0.145 2	
≥55	209	1.835 2±0.830 2	
Gender			0.211
Male	248	1.739 5±0.065 7	
Female	100	1.690 3±0.903 2	
Tumor size/cm			<0.001
≤4	120	1.827 3±0.073 8	
> 4	228	1.963 2±0.093 2	
Depth of invasion			<0.001
T _{is} -T ₁	33	0.930 2±0.293 4	
T ₂ -T ₄	315	1.129 3±0.109 3	
Lymphatic invasion			<0.001
-	192	1.039 4±0.084 3	
+	156	1.394 5±0.093 4	
Venous invasion			0.46
-	245	0.935 6±0.102 3	
+	103	1.302 3±0.132 1	
Lymph node metastasis			0.004
-	138	0.940 5±0.104 5	
+	210	1.373 2±0.095 7	
TNM staging			<0.001
I	6	0.649 5±0.203 4	
II	60	0.934 1±0.103 4	
III	104	1.234 5±0.058 4	
IV	178	1.746 6±0.043 9	
Lauren's classification			<0.001
Intestinal-type	203	0.647 3±0.080 2	
Diffuse-type	145	1.356 4±0.089 9	

(>4 cm), 浸润较深及较多淋巴结转移的胃癌组织中S100A4 mRNA呈现高表达($P<0.05$), 也支持Western blot分析S100A4蛋白的表达结果(图3, 表4)。

2.3 S100A4蛋白表达与胃癌患者生存的关系

对348例胃癌患者进行为期1个月~10.1年(中位数65.1个月)的随访。Kaplan-Meier法单因素分析显示: 弱或中度S100A4基因表达的患者累积生存率明显低于S100A4基因不表达的患者($P<0.05$)。多因素分析采用Cox比例模型来确定各种临床病理参数的独立预后作用。研究结果表明, 浸润深度($P=0.048$), 淋巴管及静脉浸润($P=0.049$ 和 0.041), 淋巴结转移($P=0.027$), TNM分期($P<0.001$)与S100A4表达($P=0.045$)均是胃癌

的预后不良的独立因素(表5, 图4)。

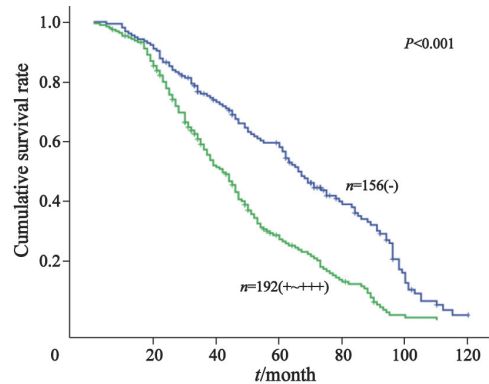


图4 S100A4蛋白的表达与胃癌患者预后的关系

Fig. 4 Correlation between S100A4 expression status and prognosis of gastric carcinoma patients

Kaplan-Meier curves were plotted to determine cumulative survival rate of patients with gastric carcinomas based on S100A4 expression. The cumulative survival rate of patients with weak or moderate

表5 胃癌患者生存的多因素分析

Tab. 5 Multivariate analysis of clinicopathological variables to determine the survival of gastric carcinoma cases.

Clinicopathological parameters	Relative risk (95%CI)	P value
Age (≥ 55 years)	1.399(0.992-1.898)	0.057
Gender (female)	0.729(0.519-1.241)	0.087
Depth of invasion (T ₂₋₄)	2.288(1.689-6.234)	0.048
Lymphatic invasion (+)	1.528(1.055-2.367)	0.049
Venous invasion (+)	1.672(1.063-2.723)	0.041
Lymph node metastasis (+)	2.042(1.114-3.931)	0.027
TNM staging (III-IV)	5.584(2.126-12.045)	<0.001
Lauren's classification (intestinal/diffuse)	1.119(0.886-1.393)	0.287
S100A4 expression (+, ++, +++)	1.522(1.325-4.236)	0.045

S100A4 expression (green curve) was lower than that of patients not expressing S100A4 (blue curve; $P<0.001$).

3 讨论

S100A4蛋白定位于胃上皮细胞的细胞质和细胞核, 这与以前的报道一致^[15-16]。S100A4是一个转录因子, 可以在某些生理或病理条件下从细胞核转运到细胞质。在本研究中S100A4 mRNA和蛋白的表达水平按以下顺序逐渐增加: 癌旁组织<慢性胃炎<肠上皮化生<腺瘤样异型增生<胃癌, 与肾透明细胞癌^[17], 食管鳞状细胞癌^[18-19], 大肠癌^[20], 乳腺癌^[21]和膀胱癌^[22]一致。肠上皮化生是一种损伤的胃上皮的自然适应状态, 根据其外观形态和生物学特性, 可发展成球样异型增生, 与印戒细胞癌的发生密切相关^[23]。病理和遗传学观察表明, 胃黏膜异型增生的发生早于胃癌, 并分为隐窝、球样再生和腺瘤亚型^[24]。有报道

称^[25-26], S100A4超表达可能导致S100A4基因第一个内含子的CpG位点发生甲基化, 或者只是在一个缺氧的微环境中S100A4刺激的结果。

通过分析S100A4的表达是否与胃癌的侵袭行为有关来阐明S100A4蛋白在胃癌进展中的作用, 本研究中S100A4 mRNA和蛋白表达与肿瘤大小、浸润深度、淋巴管和静脉侵袭、淋巴结转移和TNM分期呈正相关, 这支持Feng等^[27]以前的结果。细胞质中S100A4蛋白的表达与胃癌浸润深度和淋巴结转移呈正相关, S100A4蛋白的核表达与结肠直肠癌浸润深度和嗜神经侵袭呈正相关^[28]。晚期子宫内膜癌^[29]和膀胱癌^[30]中S100A4基因的表达和侵袭性之间呈正相关。这些结果表明, S100A4基因的超表达参与了胃癌的生长、侵袭和转移, 可作为临床实践中胃癌的侵袭性的指标。因此, 推测S100A4基因表达上调可能通过逆转癌细胞的侵犯性表型参与

胃癌的进展。

*S100A4*基因是通过下调E-cadherin表达与调控AKT/Slug通路^[19,30]，以调节人体食管鳞癌细胞的迁移和侵袭性行为。Sack等^[31]报道，*S100A4*诱导的细胞运动和转移是通过在结肠癌细胞中的Wnt/ β -catenin蛋白通路抑制剂卡西霉素所抑制的。Zhang等^[18]报道，*S100A4*蛋白介导的细胞侵袭和食管鳞状细胞癌转移是通过MMP-2和E-钙粘蛋白的活性的调节。Shen等^[32]发现，*S100A4*通过 α V和 α 5整合调节防止胃癌细胞的凋亡。Hua等^[33]发现，抑制*S100A4*蛋白促进细胞凋亡并抑制BGC823胃癌细胞在体外和体内的增殖。Xie等^[34]发现，*S100A4*介导的子宫内膜癌侵袭和诱导*S100A4*与肿瘤生长因子(TGF)- β 1信号传导的Smads蛋白激活相关。基于这些数据和我们的研究结果，推测*S100A4*通过Wnt信号/ β -catenin蛋白或TGF- β 信号通路上调，增强了侵犯性的表型和恶性肿瘤的行为。

虽然胃癌起源于相同的胃上皮细胞，但由于患者的个体差异，其形态特征大不相同。根据Lauren分型，肠型胃癌以凝聚的肿瘤细胞所构成的腺样管状结构为特征，呈膨胀或浸润性生长，包括高、中分化腺癌^[7]。相反，弥漫型胃癌则以不明显的腺癌或缺乏细胞黏附为主要特征，包括低分化和印戒细胞癌^[23]。在本研究中，*S100A4*的蛋白和mRNA在肠型中表达明显低于弥漫型，表明*S100A4*可能在其中发挥了重要致癌作用，可作为这两种类型胃癌之间区别的分子基础。

在本研究中，*S100A4*基因的表达与胃癌患者预后良好呈负相关，这与以前的报道相一致^[15-16]。Cox比例风险分析表明，浸润深度，淋巴管或静脉侵袭，淋巴结转移，TNM分期和*S100A4*表达是胃癌患者预后不良的独立因素。Kho等^[35]发现，C期结肠癌患者只有细胞质出现*S100A4*的染色，细胞核不染色，*S100A*表达与患者的预后不良相关。Kang等^[28]认为大肠癌患者细胞核*S100A4*蛋白的表达是预后不良的独立因素。类似的结果也出现在胰腺癌^[36]、

肾细胞癌^[37]、膀胱癌^[22]等。这些结果表明，*S100A4*基因表达是胃癌患者预后不良的独立和可靠的指标。

综上所述，*S100A4*基因的上调可能在胃上皮细胞的恶性转化中起重要作用，与胃癌的生长、侵袭、转移和不良预后呈正相关。*S100A4*可被视为胃癌侵犯行为的一个有力的标志物。*S100A4*在弥漫型癌的独特表达可以用来区分肠型癌和弥漫型癌。

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